Function of the Sodium Pump in Arterial Smooth Muscle in Experimental Hypertension: Role of Pressure

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SUMMARY Several laboratories have reported evidence suggesting that in hypertension there are abnormalities in the activity of the sarcolemmal sodium pump in vascular smooth muscle. In the present study, by sampling tissue from both hypertensive and normotensive portions of the arterial tree in coarctation hypertension, we investigated the relationship of such abnormalities to the elevated intravascular pressure. Additionally, we measured plasma renin activity and body fluid volumes. We assessed sodium pump activity in vitro in sodium-loaded arteries freshly excised from rats with chronic coarctation hypertension (abdominal aorta constricted above the renal arteries 4 to 5 weeks previously) and from normotensive sham-coarcted rats; these included the tail artery, the hypertensive thoracic aorta, and the normotensive portion of the abdominal aorta below the clip. The $^{86}$Rb uptake in the absence (total uptake) and presence (ouabain-insensitive uptake) of 1.0 mM ouabain was measured, and ouabain-sensitive uptake (nmole/mg dry weight/10 min) was calculated. In the rats with coarctation hypertension, there were significant increases in the ouabain-sensitive and total $^{86}$Rb uptakes in both the thoracic and abdominal aorta, but no abnormalities in $^{86}$Rb uptake in the tail artery. The higher uptakes in tissue from coarcted rats could not be attributed to increased levels of intracellular sodium. Plasma renin activity was elevated in the coarcted rats, but no changes in body fluid volumes were detected. This study provides evidence that increases in the activity of the sodium pump, similar to those we have observed in tissue from rats with salt-induced or Goldblatt hypertension, occur in conduit arteries of rats with coarctation hypertension studied in vitro. Because similar increases occur in vascular smooth muscle of the normotensive and hypertensive portions of the aorta, these pump abnormalities in arteries do not appear to be the direct result of increased intravascular pressure and probably are not attributable to tissue hyperplasia.

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KEY WORDS • arterial hypertension • tail artery • aorta • Na, K-ATPase • plasma volume • blood volume • extracellular fluid volume

In recent in vitro studies of conduit arteries from rats with salt-induced or Goldblatt hypertension, we found evidence for increases in activity of the sarcolemmal sodium pump of the vascular smooth muscle. These pump abnormalities did not appear to be related to changes in body fluid volumes, to increases in intracellular concentrations of sodium, or to altered activity of the renin-angiotensin-aldosterone system. Our present studies were designed to determine whether these arterial changes might result in some way from the elevated intraarterial pressure. Coarctation hypertension is a model allowing study of arteries not exposed to elevated intravascular pressures. We have had considerable previous experience with this form of experimental hypertension. In our present experiments we measured in vitro $^{86}$Rb uptake by the hypertensive portion of the aorta (thoracic), the normotensive portion of the aorta (abdominal), and also the tail artery freshly excised from rats with chronic coarctation hypertension and from normotensive sham-coarcted rats.
Methods
Normotensive male Sprague-Dawley rats approximately 2 months old and weighing 150 to 200 g were randomly divided into two experimental groups. To create coarctation hypertension, we placed a partially constricting silver clip (diameter 0.813 mm) around the abdominal aorta upstream to the origin of both renal arteries. In control rats, a clip (diameter 1.7 mm) too large to constrict the aorta was similarly placed. Postoperatively, the rats were maintained on a diet of standard rat chow (Na+ 0.39%, K+ 0.96%) and tap water ad libitum.

To document that the hindquarters vascular bed of the coarcted rats remained normotensive, we cannulated the abdominal aorta, via the femoral artery, in four coarcted hypertensive and three control normotensive sham-coarcted rats 2 weeks after clipping and in an additional three coarcted and two control rats 4 weeks after clipping. The rats were tethered and allowed to recover from ether anesthesia. Then, we continuously monitored pressures (by Statham pressure transducer and Hewlett-Packard recorder) in the conscious animals for periods ranging from 6 to 20 hours and in both light and dark environments. No increased lability of pressure was detected in the coarcted rats. Thus, pressures were read every 30 minutes and means calculated and compared by unpaired Student's t test. The null hypothesis was rejected at p<0.05.

In other similarly prepared hypertensive and paired control rats 4 to 5 weeks after surgery, we measured carotid and femoral arterial pressures directly under pentobarbital anesthesia (45 mg/kg, i.p.). After administering additional pentobarbital (15 mg/kg, i.p.), we then obtained arterial tissue from these rats for measurement of Rb uptakes. The ventral tail artery, then the abdominal aorta downstream to the renal arteries (and 1.5 to 2 cm downstream from the clip), and, finally, the descending thoracic aorta were gently and rapidly excised from each of the paired rats in turn. Placed in Krebs-Henseleit solution (NaHCO₃ 27.2 mM; NaCl, 117.0 mM; K₂HPO₄, 1.0 mM; KCl, 4.8 mM; MgSO₄·7H₂O, 1.2 mM; CaCl₂·2H₂O, 1.25 mM; and glucose, 11.1 mM) at 27°C bubbled with 95% O₂, 5% CO₂ (pH 7.4), these specimens were gently and rapidly (<5 min) cleaned of adventitia and blood and opened longitudinally. Then, following a sodium-loading procedure, Rb uptakes were immediately measured in the arterial tissue from the paired rats by procedures similar to those we have previously reported.¹,² In brief, the arteries from the two rats were placed in K⁺-free Krebs-Henseleit solution (NaHCO₃ 27.2 mM; NaCl, 117.0 mM; NaH₂PO₄·H₂O, 1.0 mM; MgSO₄·7H₂O, 1.2 mM; CaCl₂·2H₂O, 1.25 mM; and glucose, 11.1 mM) at 0 to 2°C for 5 minutes of sodium-loading. Next, the arteries were incubated for 10 minutes at 37°C in an O₂/CO₂ bubbled K⁺-free Krebs-Henseleit solution containing "cold" RbCl, 2 mM, plus trace amounts of ⁸⁶RbCl (New England Nuclear). For this incubation, each artery was divided in half. One-half was incubated in the medium without ouabain and the other half incubated in the medium with added ouabain (1.0 mM). Tissues were then washed three times (total time 15 to 20 seconds) with 0°C K⁺-free Krebs-Henseleit containing 2 mM "cold" RbCl, blotted with tissue paper to remove surface fluid, weighed, and placed in a crystal scintillation counter to determine ⁸⁶Rb uptake. The tissue was then dried at 100°C for 24 hours and reweighed. The ⁸⁶Rb uptake was calculated as nmole/mg of wet weight/10 min and also as nmole/mg of dry weight/10 min. Ouabain-sensitive uptake was calculated as the difference between the ⁸⁶Rb uptake without (total uptake) and with (ouabain-insensitive uptake) ouabain. Student's t test for paired replicates was used for statistical analysis.

In an additional 12 rats with coarctation hypertension and 12 sham-coarcted rats prepared identically to those described above, we investigated the relationship of intracellular sodium in the aortic tissue to the ⁸⁶Rb uptakes described above. Thoracic aorta was excised from these rats, cleaned of adventitia and blood, opened longitudinally, and sodium-loaded for 5 minutes exactly as described above. Half of each artery was then loaded for 40 minutes with lithium at 0 to 2°C for estimation of cellular sodium content, as previously described.¹,² The other half was incubated for 10 minutes in K⁺-free Krebs-Henseleit with 2 mM "cold" RbCl, exactly as described above, except that ⁸⁶RbCl and ouabain were omitted. After incubation, these tissues were also immediately lithium-loaded for estimation of cell sodium. Means of values in coarcted and sham-coarcted rats were compared by unpaired Student's t test.

In nine other rats with coarctation hypertension and nine paired sham-coarcted rats, we measured plasma renin activity by radioimmunoassay (Angiotensin I Immutope Kit, E. R. Squibb and Sons, Inc.). For this measurement, trunk blood was collected for the first 3 seconds following decapitation of conscious rats. Finally, body fluid volumes were measured in six similar rats with chronic coarctation hypertension, 28 similar age-matched rats with sham coarctation, and eight weight-matched rats that had had laparotomy (with or without unilateral nephrectomy) but no clipping. Volumes were measured at the same time of day in each group to control for diurnal variation. Methods used were similar to those we have previously reported.¹,² Briefly, with the rat under ether anesthesia, both kidneys were excised by flank incision. Then approximately 0.05 μCi ¹²⁵I-labelled human serum albumin and 0.05 μCi Na₂⁴NaSO₄ were injected intravenously in 100 μl saline, with a flush of 300 μl saline. For standards, identical injections were made into volumetric flasks and diluted. To facilitate complete mixing of the labels, the rats were allowed to recover from the anesthesia. Then, exactly 1 hour after the isotopes were administered, the conscious rats were decapitated and trunk blood collected for hematocrit and isotope concentrations. The ¹²⁵I was measured in 50 μl aliquots of plasma and standards on a crystal scintillation counter; plasma volume and total blood volume (hematocrit corrected for 4%...
trapped plasma) were calculated. Plasma proteins were then precipitated with ice-cold 10% trichloroacetic acid. The 35S was measured in 50 μl aliquots of deproteinated plasma and standards in a liquid scintillation counter; extracellular fluid volume was thereby calculated. Later counting of these deproteinated specimens in a crystal scintillation counter revealed that less than 3% of the 35S remained, verifying the removal of most albumin by trichloroacetic acid and also verifying that dissociation of the 35S from the albumin was less than 3%. Measured body fluid volumes were plotted against body weight and regression lines, and 95% confidence intervals were plotted for pooled values in the age- and weight-matched normotensive control rats. Values in the coarcted rats were compared against these intervals.

At necropsy, general health was evaluated. Heart, and, in some rats, kidney, and adrenal weights, were measured for calculation of organ weight/body weight. In six pairs of rats, serum was obtained for measurement of creatinine concentrations (Autoanalyzer). (We have ample previous evidence that serum creatinine and electrolyte concentrations remain within normal ranges in our rats with chronic coarctation hypertension.6)

**Results**

Table 1 presents the mean values (± SEM) of final body weight, arterial blood pressures directly measured under light pentobarbital anesthesia 4 to 5 weeks after clipping, heart weight/body weight, kidney weight/body weight, and adrenal weight/body weight in the pairs of rats in which arterial uptakes of 86Rb were measured, and also presents serum creatinine concentrations measured in six pairs of rats. Significant hypertension developed in the coarcted rats, accompanied by slightly decreased body weight and increased heart weight/body weight. There were no significant differences between hypertensives and normotensives in kidney weight/body weight or serum creatinine levels. Plasma renin activity was approximately doubled in the rats with coarctation hypertension, accompanied by significant increases in adrenal weight/body weight. All rats were found at autopsy to be in good general health.

Table 1 also presents femoral arterial pressures directly measured in these coarcted and sham-coarcted rats under pentobarbital anesthesia. No significant differences were detected (p > 0.2). Similarly, continuous direct pressure measurements in the abdominal aorta made for periods of 8 to 20 hours in conscious rats indicated equal levels in the coarcted and sham-coarcted groups. Two weeks following clipping or sham-clipping, abdominal aortic pressures were (mm Hg; mean ± SEM): 94 ± 1 in sham-coarcted (n = 3), and 91 ± 1 in coarcted (n = 4) rats. Four weeks after clipping or sham-clipping, pressures were 93 ± 1 in sham-coarcted (n = 2), and 85 ± 1 in coarcted (n = 3) rats.

Table 2 presents in vitro 86Rb uptakes by thoracic aorta, abdominal aorta, and tail artery. As indicated, paired Student’s t test revealed that the total, ouabain-insensitive, and ouabain-sensitive uptakes were elevated in thoracic aortas of rats with coarctation hypertension. Increases of similar magnitude in the total and ouabain-sensitive components, but not in the ouabain-insensitive component, occurred in the normotensive abdominal aortas from these rats with coarctation hypertension. In tail artery, in contrast,

<table>
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<tr>
<th>TABLE 1. Body Weights, Carotid and Femoral Artery Mean Pressures, Organ Weights/Body Weights, and Serum Creatinine (Means ± SEM) in Paired Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body wt</strong>&lt;br&gt;(g)</td>
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<tr>
<td>433.5 ± 5.0</td>
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<tr>
<td>354.3 ± 3.7</td>
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<tr>
<td>132.8 ± 3.7</td>
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<tr>
<td>27.1 ± 0.4</td>
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<tr>
<td>35.3 ± 0.7</td>
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<tr>
<td>34.6 ± 0.6</td>
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<tr>
<td>1.38 ± 0.05</td>
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<tr>
<td>0.87 ± 0.02</td>
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<td>3.26 ± 0.63</td>
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</tbody>
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*Student’s t test for paired replicates.

<table>
<thead>
<tr>
<th>TABLE 2. 86Rb Uptake (nmole/mg dry weight/10 min; mean ± SEM)</th>
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</thead>
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<tr>
<td><strong>Thoracic aorta</strong>&lt;br&gt;No.</td>
</tr>
<tr>
<td>28</td>
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<tr>
<td>28</td>
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<tr>
<td>43.0 ± 1.7</td>
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</table>

*Student’s t test for paired replicates.
there were no significant differences in uptakes between hypertensive and normotensive control rats. Calculations made using wet weights, or using the unpaired Student's $t$ test, led us to similar conclusions.

Lithium substitution methods were used to estimate cellular sodium content in thoracic aorta excised from 12 sham-coarcted and 12 coarcted rats. Following 5 minutes of sodium loading in potassium-free Krebs-Henseleit solution, sodium content, in mmoles/kg dry solid, was $31.8 \pm 2.2$ in sham-coarcted and $36.4 \pm 2.1$ in coarcted rats ($p > 0.1$). Following 5 minutes of sodium loading plus 10 minutes incubation in K+-free Krebs-Henseleit solution with 2 mM RbCl at $37^\circ$, sodium content was $44.0 \pm 2.3$ in sham-coarcted and $48.4 \pm 2.1$ in coarcted rats ($p > 0.1$).

Measured body fluid volumes plotted against body weight are presented in figures 1–3. Regression lines and 95% confidence intervals in these figures were plotted for pooled values in the age- and weight-matched normotensive control rats. (Values in unilaterally nephrectomized control rats did not differ from those in control rats with intact kidneys.) It may be seen that body fluid volumes in most rats with coarctation hypertension fell within these 95% confidence intervals, providing little or no evidence that altered body fluid volumes accompany this form and stage of coarctation hypertension. Hematocrits in the groups of rats in which body fluid volumes were measured did not differ significantly (mean ± SEM: $44.3 \pm 2.2$ vol% in hypertensives; $43.8 \pm 0.6$ vol% in normotensive controls).

**Discussion**

Studies of resistance vessels in vivo in various forms of hypertension and in several species have provided evidence suggesting decreased activity of the sodium pump of the sarcolemma of vascular smooth muscle. In contrast to these in vivo studies, most laboratories have reported increases in pump activity in conduit arteries (tail artery, aorta) excised from rats with various forms of experimental hypertension and studied in vitro. Both findings are of interest because their basic mechanisms are probably interrelated and involved in the pathogenesis of hypertension.

Our previous in vitro studies of rat conduit arteries have provided evidence that these increases in pump
activity are not related to volume-expansion,1,2 to activity of the renin-angiotensin-aldosterone system,3 or to increases in intracellular Na+ concentration.1,2 In the present project, we investigated the possibility that they may be caused, in some way, by the elevated intraarterial pressure.

We have had considerable previous experience with coarctation hypertension in rats, the model of experimental hypertension we used in the present study. We and others have documented, by indirect and direct measurements of arterial pressures in both conscious and anesthetized rats, that the hindquarters vascular beds remain normotensive. In the present study, we used indwelling cannulas to monitor hindquarters pressures for hours in conscious coarcted rats at several stages in the development of the hypertensive state and found no evidence for even transient elevations in arterial pressure. Thus, there is no evidence that the abnormalities observed in these normotensive beds are merely the effects of the elevated intrarterial pressures. In these normotensive vascular beds of animals with experimental coarctation hypertension, we and others have found evidence for vascular wall "waterlogging," increases in vascular wall weight, increases in tritiated-thymidine labeling, and elevations in the structural and neurogenic components of peripheral vascular resistance.4,5,6,7

We also previously observed increased activity of the renin-angiotensin-aldosterone system in chronic coarctation hypertension in rats,4 and the present study confirms this finding. The present study additionally provides new evidence that the chronic stage of this form of experimental hypertension may not be accompanied by changes in body fluid volumes. These findings would suggest that coarctation of the aorta above the renal arteries in rats produces a chronic hypertensive myopathy similar to two-kidney, one clip than to one-kidney, one clip Goldblatt hypertension.

As in our previous investigations,1,2 we interpreted the ouabain-sensitive component of 86Rb uptake by arterial tissue as a measure of sodium pump activity in the sarcolemma of the smooth muscle cells. As previously, we noted that pump activity per unit tissue weight was higher in the thoracic aorta than in the tail artery. We found intermediate values in tissue from the abdominal aorta. These differences in pump activity per unit weight in the various arterial segments are likely related to differences in wall composition, i.e., differences in the relative masses of smooth muscle and connective tissue as well as differences in diffusion distances and barriers.

The present study also provides new evidence that abnormalities exist in sodium pump activity in vascular smooth muscle in coarctation hypertension. The increases we observed in the ouabain-sensitive component of 86Rb uptake in aortic tissue are similar to those we previously found in Dahl salt-sensitive hypertensive rats1 and in rats with both one-kidney, one clip and two-kidney, one clip Goldblatt hypertension.2 Whether abnormalities also occur in resistance vessels in vivo in coarctation hypertension requires further investigation.

Regarding the role of intraarterial pressure in producing these increases we observed in pump activity in conduit arteries, our data are more difficult to interpret. Neither the abdominal aorta nor the tail artery in our rats was exposed to elevated pressure. Thus, we would have expected to find similar results in these two vessels. In contrast to the elevated sodium pump activity in abdominal aorta, however, there was no evidence for abnormal pump activity in the tail artery. It should be noted, though, that coefficient of variability of 86Rb uptake was greater in the latter.

Even with this reservation, we conclude that our observation of increased pump activity in the smooth muscle of the abdominal aorta provides good evidence that such abnormalities are not the direct result of elevated intraarterial pressure.

We have previously1,2 suggested that, among other explanations, the increases observed in sodium pump activity in vascular tissue in hypertension may reflect more sarcolemma per unit tissue weight, due, for example, to hyperplasia of the vascular smooth muscle. In our previous studies of coarctation hypertension in rats we found evidence for increases in weight of the normotensive abdominal portion of the aorta.8 The magnitude of these increases, however, was only 40% of the increases we observed in the hypertensive thoracic aorta. In rabbits with coarctation hypertension, Bevan observed significant increases in the H-thymidine labeling index in the normotensive abdominal aorta. Again, the magnitude of these increases was only about 20% of the increases observed in thoracic aorta. Nevertheless, these data are sufficient to suggest that a degree of vascular hyperplasia may occur even in normotensive vascular beds in coarctation hypertension. Therefore, we cannot rule out the possibility that the increases in ouabain-sensitive Rb uptake we observed in abdominal aorta are attributable, at least in part, to vascular smooth muscle hyperplasia. However, the results of our present studies would argue against this explanation, because average percent increase in ouabain-sensitive Rb uptake (table 2) was greater in abdominal (+24%) than in thoracic aorta (+14%). Nevertheless, further studies are necessary to resolve this possible explanation.

It has also been suggested that such increases in vascular pump activity may result from increased membrane leakiness to Na+.14,15,19 elevating intracellular sodium. However, in the present study there was little or no evidence for increased intracellular sodium in vascular smooth muscle cells of the coarcted rats. We previously observed that, even with considerable cell sodium-loading, the sodium pumps of smooth muscle cells of control normotensive rats do not operate at the high levels we observe in cells of hypertensive (Dahl salt-sensitive; Goldblatt) rats.1,2 Thus, vascular muscle from the hypertensive rats apparently does not function simply like sodium-loaded muscle from control rats. In ongoing studies (T. A. Brock, unpublished observations), we have observed a sigmoid relationship between total cellular sodium and ouabain-sensitive Rb uptake by rat aortic tissue, similar to that reported for erythrocytes.20 However, for each level of...
intracellular sodium (adjusted over a wide range by incubation techniques and use of the ionophore, monensin), we found greater pump activity in vascular tissue excised from rats with hypertension (DOCA-salt or one-kidney Grollman). Based on this evidence, we feel it is unlikely that the increases in electrogenic ion transport that have been observed in arterial tissue in certain forms of hypertension merely reflect increased intracellular sodium concentration in the vascular smooth muscle cells. Because increases in pump activity were found in the normotensive abdominal aorta in the present study, the present data further allow us to argue that such increases in pump activity are not related to pressure-dependent increases in smooth muscle membrane permeability to sodium ion.10

Another explanation may be that the increased pump activity reflects a faster turnover rate of a normal complement of pump molecules that is apparently not related to [Na+]o, for reasons stated above, nor to [K+]i, because levels of K+ in the incubating media were controlled, nor to activity of the renin-angiotensin-aldosterone system.2 Changes in vascular wall content of other chemical agents influencing pump activity, such as prostaglandins,11 might be involved. It is also possible that metabolic differences allowing greater resistance to hypoxia during in vitro studies, or higher levels of high energy phosphates in the tissues from the hypertensive rats, may be involved, although there is little data to support this contention.

Although we do not have direct evidence to support it, we favor another explanation, that there are more pump molecules per unit sarcolemma in the vascular smooth muscle from the hypertensives, perhaps due to induction by a circulating digitalis-like pump inhibitor24-28 that, in rat tissue, dissociates from the pump molecules during the in vitro studies.

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